Extension of green bell pepper shelf life using oilseed-derived lipid films from soapstock☆

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ARTICLE INFO

ABSTRACT

Edible films have been used for decades on fresh produce to create a semipermeable membrane on the surface to suppress respiration, control moisture loss, and more recently to provide a delivery mechanism for the inclusion of functional components. Scientists at the Southern Regional Research Center (SRRC) have previously demonstrated that a thin biodegradable film can be produced from soapstock, an underused byproduct from the vegetable oil industry. After physical and chemical treatments, a thin film was produced from various soapstocks (cottonseed and safflower). Different hydration ratios were tested since the initial soapstock solutions were rather viscous. To examine the potential use of an oilseed-derived lipid film for the extension of shelf life, different types of the oilseed-derived soapstocks were utilized to produce lipid films with different hydration ratios, and containing 0, 5, and 10% of paraffin wax for application on 'Camelot' bell peppers. Control bell peppers lost almost 25% weight per unit surface area (SA) in 78 h when stored under ambient conditions. Cottonseed film-coated peppers, hydrated at 1:4, lost only about 5% moisture per unit SA after 78 h and minimized weight loss by up to 79% compared to the control. However, since a 1:4 hydration ratio remained rather viscous, 1:8 was preferred and these cottonseed films reduced weight loss per unit SA by up to 48% during storage. Safflower-derived soapstock film resulted in the least effective water retention of the films and ratios tested, with roughly 21–25% reduction in weight loss per SA compared to controls. Safflower-derived soapstock was higher in unsaturated fatty acids, which are less efficient to control moisture migration because they are more polar than saturated lipid materials, as contained in cottonseed-derived materials. Addition of wax to the cottonseed-derived films decreased water loss slightly, similar to previous reports in the literature. An ANOVA supported the conclusion that the oilseed-derived lipid films significantly reduced moisture loss across the produce epidermis. To avoid potential allergenicity concerns in cottonseed soapstock, additional cleanup steps and tests with commonly used edible coating additives would be required before attaining food grade status.

Published by Elsevier B.V.

1. Introduction

Wax coatings have been used since the 1930s to protect and extend shelf life of various fruits and vegetables (Hardenburg, 1967; Kester and Fennema, 1986). Edible films and waxes have been used for decades on fresh produce to create a semipermeable membrane on the surface to suppress respiration, control moisture loss, add gloss, and more recently, to provide a delivery mechanism for additional functional components (Hardenburg, 1967; Kester and Fennema, 1986; Min and Krochta, 2005). Wax coating applications to fruits and vegetables such as apples, cucumbers, citrus, rutabagas and tomatoes were common, and are still commonly practiced (Hitz and Hout, 1939; Jones and Richey, 1939; Paredes-López et al., 1974; Hagenmaier and Baker, 1994; Kays, 1997). Alternatives to wax have been explored with edible films (Kester and Fennema, 1986).

Moderate water barrier properties have been reported in mechanically abused brownies using films containing lipids and hydrocolloids (Greener and Fennema, 1989). An edible film, composed of straight-chained fatty acids made of carbons between 12 (lauric) and 18 (stearic), had a good characteristic to be used as a shelf life extender, even applicable to cereal products (Hagenmaier and Shaw, 1990). Utilization of protein-based lipid films has been reported for extending shelf life of vegetables such as carrots,

cucumber and bell peppers (Gauoth et al., 1991; Avena-Bustillos et al., 1994).

In processing oil-bearing materials, free fatty acids (FFAs) are extracted with the main components of edible oil, triacylglycerols. These FFAs are fractioned and separated from the edible oil by titration with caustics, producing a byproduct, soapstock. In the past decades, soapstock has been recovered as raw materials for producing low grade industrial soaps. After vegetable oil production, typical oilseed (soy, cottonseed and safflower) byproducts like cake and/or soapstock may be used in animal feeds. Because of low economical return, most soapstock is rarely recovered today, but added back to animal feed without much economic compensation (Matthäus and Zubr, 2000). Most commercial seed oils and resulting cakes are unlike certain seeds possessing high quantities of antioxidants that warrant commercial extraction of key phytonutrients (Peschel et al., 2007). Alternative byproducts such as lipid film constituents (Kuk and Ballew, 1999) or biodiesel production (Haas et al., 2001, 2006; Dumont and Narine, 2007) have been explored, and may therefore be warranted.

In general soapstock is mostly made of FFA with minor components including glycerol, acylglycerides, sterols, phospholipids, phenolics and their degenerated compounds (Dowd, 1996, 1998; Kuk and Ballew, 1999). With application of a few physical/chemical treatments to these oilseed lipids, a bilayer film of oilseed lipids can be produced by hydrating the materials produced from oilseed soapstock (Kuk and Ballew, 1999). Because of the unique chemical compositions, most soapstocks have a property necessary for structural facilitation known as "mesomorphism" (Small, 1986). Lipid films developed from various oilseeds were postulated to have potential postharvest application as a shelf life extender for produce. Freshly harvested bell peppers were therefore tested to evaluate the potential of preliminary oilseed-derived lipid films from soapstock as shelf life extenders. Retention of fresh weight in bell peppers with and without various oilseed-derived film coatings is reported.

2. Materials and methods

2.1. Chemical properties of lipid/wax film

Soapstock samples of cottonseed and safflower seed oils were obtained from industrial plants. The capillary gas chromatography (GC) composition analyses of the soapstocks after trimethylsilyl derivatization (TMS) were previously reported (Kuk and Ballew, 1999), according to GC analyses presented elsewhere (Dowd, 1996). Typical ranges of these analyses are summarized in Table 1. The typical properties (volatiles, neutral oil and fatty acids) were determined by the AOCS (American Oil Chemists Society) official and recommended methods. The elemental analysis (phosphorus and nitrogen) of the soapstock material was done by ASTM D5373/E258. The chemical composition analysis was done by a high temperature capillary GC (Hewlett-Packard 5890, Avondale, PA) with a TMS technique using the AOCS method Cd.11b-91. A thin layer chromatography method with a flame ionization detector was used to determine phospholipids in the film material (St.Anjelo and James, 1993). The Iatroscan TH-10 TLCEFID Analyzer was interfaced with a Hewlett-Packard (Palo Alto, CA) 3390 Integrator. The FID was operated with a hydrogen gas flow of 160 mL/min. Air was supplied via an electrical air pump. The air flow was 2 L/min. Separations were performed on silica-coated Chromarods S-III (Iatron Labs., Inc.). Until initial use, the rods were stored in 60% sulfuric acid. Rods were thoroughly washed with distilled water immediately prior to use and scanned twice to burn off impurities that may have remained. After spotting rods with extracts from experimental samples and scanning with the Iatroscan TLC/FID Analyzer, they were routinely burned again and then stored in a 100% humidity chamber until

Table 1
Chemical and elemental composition of cottonseed and safflower soapstocks.

Chemical classes (% dry basis)	Soapstocks	
	Cottonseed	Safflower
Total fatty acids	50–55	50–63
Neutral oils	15–22	11–15
Saturated fatty acids[a]	21	12
Unsaturated fatty acids	41	50
Phosphorus	0.5–0.6	0.9–1.1
Nitrogen	0.5–0.7	0.7–1.0
Sucrose	Trace	1.9
Oligosaccharides[b]	0.8	1.2
Sterols[c]	2.9	2
Monoglycerides	1.8	1.3
Diglycerides	2.6	1.5
Triglycerides	11.5	10.5

[a] Saturated fatty acids included myristic, palmitic, stearic, arachidic acids. Unsaturated fatty acids included palmitoleic, oleic, and linoleic acids. Official AOCS methods were used per Section 2 and Firestone (1993).
[b] Oligosaccharides included raffinose and stachyose.
[c] Sterol mixture included β-sitosterol, campesterol and stigmasterol.

the next analysis (St.Anjelo and James, 1993). As a supplementary analysis, the amount of FFA in crude oil samples was determined by AOCS method G 5a-40 (Firestone, 1993).

2.2. Soapstock treatments for lipid films

Soapstocks were used to prepare the oilseed-derived lipid films via the following physical treatments: 1. Freeze drying (overnight at −40 °C; 40 mm Hg), 2. particle size reduction (passable through 35 US sieve), 3. pH reduction (neutralization) and hydration, and 4. casting and drying. The film preparation steps were essentially the same as previously reported (Kuk and Ballew, 1999) with exception of the neutralization step. In general the industrial soapstock samples had pH values between 9 and 11. The soapstock samples were neutralized to a pH between 6.5 and 7.5 using diluted 5% sulfuric acid (J.T. Baker, Phillipsburg, NJ). These soapstock materials were then hydrated (soapstock:de-ionized water ratios of 1:4 and 1:8), and some were amended with 0, 5 and 10 wt.% paraffin wax (Sigma–Aldrich, Milwaukee, IL) with a melting point of 65 °C.

2.3. Produce

'Camelot' green bell peppers (161.5 g average) were collected in the middle of yearly harvest from a Louisiana farm, washed with de-ionized water, air dried, and inspected on the same day of collection to assure that samples were free from skin damage and fungal infestation. The dimensional specifics (length and radius), weight and volume were recorded. The surface area of the bell pepper samples was computed using a cylinder with hemisphere as the model.

The bell pepper samples were divided into several groups which were used to apply tested films including control, cottonseed at 1:4 and 1:8 hydration ratio, cottonseed film at 1:8 with 5% paraffin wax, safflower at 1:8 hydration, and safflower film hydrated to 1:8 receiving 10% paraffin wax. A single coat of the soapstock-derived film was uniformly applied to the samples using a fine paint brush initially, then sprayed on with a pressurized sprayer. The same coat was applied to a thin glass plate to examine and assure the uniformity of the film. Film thickness was measured using a micrometer. Microscopic examinations were conducted when necessary.

To purposely accelerate weight loss and rapidly assess film coating effects, control and the coated samples were stored in open air for up to 10 days in a laboratory at 21–24 °C with 60–75% relative humidity. Elevated storage temperatures were chosen to hasten moisture loss, and also mimic short-term local farmer's market conditions. An analysis was also performed after allowing
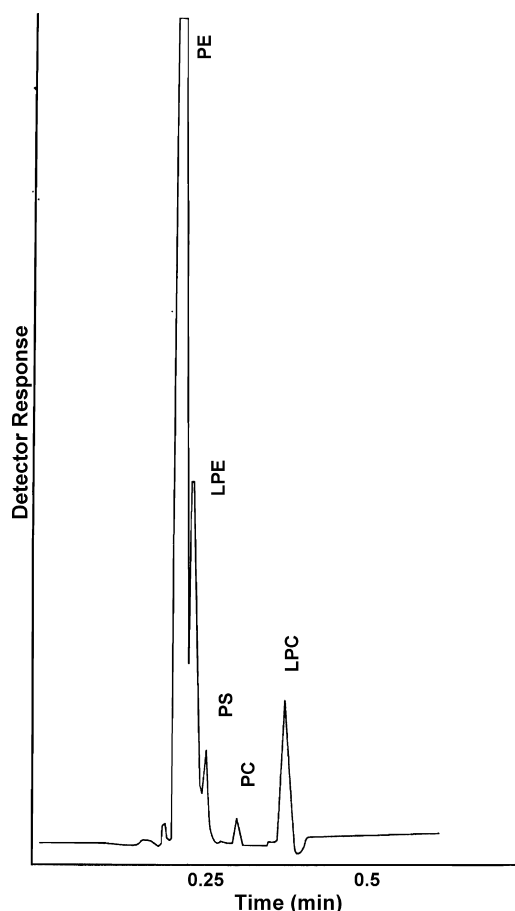
Fig. 1. An Iatroscan thinlayer chromatogram of phospholipids in cottonseed soapstock-derived film. Abbreviations relate to phosphatidylcholine (PC), phosphatidyl-ethanolamine (PE), phosphatidyl-serine (PS), lyso-PC (LPC), and lyso-PE (LPE).

fruit to remain at ambient temperatures for 2 days prior to applying soapstock-derived lipid films. Pepper weights were periodically recorded along with visual examination of the sample epidermis.

3. Results and discussion

The amount of free fatty acids (FFA) in crude oil samples were roughly 2–3 and 0.5% for cottonseed and safflower, respectively (data not shown). However, FFA (saturated and unsaturated) comprised roughly 55–60% for both soapstocks (Table 1). The film contained water-swelling amphiphiles (Small, 1986) such as phosphatidylcholine (PC) and phosphatidyl-ethanolamine (PE). The film also contained lyso-PC (LPC), lyso-PE (LPE) and phosphatidyl-serine (PS) (Fig. 1). Some of these compounds were reported to form a film (Gilbert, 1985; Kester and Fennema, 1986), but collectively the film forming characteristics of these lipids were not reported. From the phosphorus content (Table 1 and Fig. 1), one may postulate that 20–30% of phosphatides in the soapstocks contributed to a significant physical property of the freeze-dried lipids. A multiplying factor of 25 is used to estimate the amount of phosphatides from the phosphorus content (Marmer, 1985). Subsequently, this assumption is plausible considering the phosphorous content (Table 1) results in functional changes in protein film gel forming abilities resulting from different protein sizes and disulfide bond relationships with polymerization, analogous proteins (Hoshi et al., 1982; Cho and Rhee, 2004). The results of chemical composition analysis by capillary GC indicated that the following compounds were in the film: free fatty acids and glycerol, acylglycerols, saccharides,
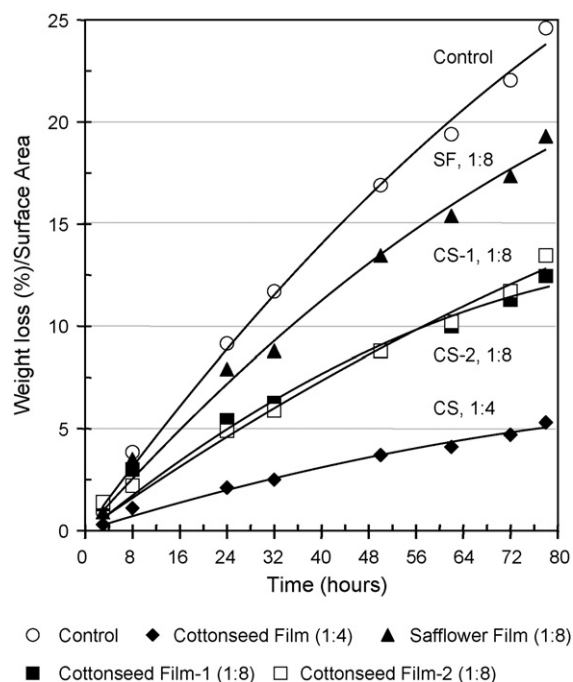
Fig. 2. Weight loss (%) per unit surface area (SA) in 'Camelot' bell peppers coated with oilseed-derived lipid films with different hydration ratios.

plant sterols and degenerated acylglycerols (Table 1). The fatty acids included saturated and unsaturated acids with carbon number between 14 and 18 (myristic, palmitic, oleic and linoleic) and mono-, di-, and triacylglycerols composed these fatty acids. The fatty acids, glycerides, and minor sugars and sterol composition of these soapstock materials essentially had an insignificant variation.

When hydrated, it was found that the treated soapstock materials made a gel that formed an enclosing or encapsulating surface upon drying. Different hydration ratios were tested since the original soapstock solutions were rather viscous. Regardless of the film type and hydration composition, resultant films were of uniform thickness (0.25 mm), and withstood a force of roughly 68.9 Pa, as previously reported (Kuk and Ballew, 1999). The film was ductile and stable such that a uniform surface was maintained even when applied to a sphere model of glass. An advantage to using soapstock-derived lipids is that gums and emulsifiers are not required to produce coatings (Conforti and Ball, 2002; Conforti and Zinck, 2002). We too observed that slight changes in crystallization occurred due to temperature fluctuations during casting (data not shown), and we attribute this mainly to slight differences in sucrose and saccharide content (Table 1). This appears analogous to temperature effects summarized for starch film crystallization due to varying amylose content (Myllärinen et al., 2002). We subsequently reported data for a replicated film so as to verify consistency between batches of soapstock-derived films (Fig. 2).

The mean weight loss per unit surface area (SA) versus time in bell peppers receiving cottonseed and safflower soapstock films indicates clearly that water loss was restricted substantially (Fig. 2). Control bell peppers lost almost 25% weight per unit SA in 78 h (Fig. 2). The polynomial equation describing this loss was: $y = -0.0012x^2 + 0.3985x$ ($R^2 = 0.996$). Cottonseed film hydrated at 1:4 lost only roughly 5% moisture per unit SA after 78 h and minimized weight loss by up to 79% compared to the control. However, since a 1:4 hydration ratio resulted in rather viscous coatings, a 1:8 hydration ratio was preferred. The 1:8 ratio cottonseed films reduced weight loss per unit SA by up to 48% during storage. A clear trend and pronounced effect between the control group and the ability for cottonseed films to restrict water
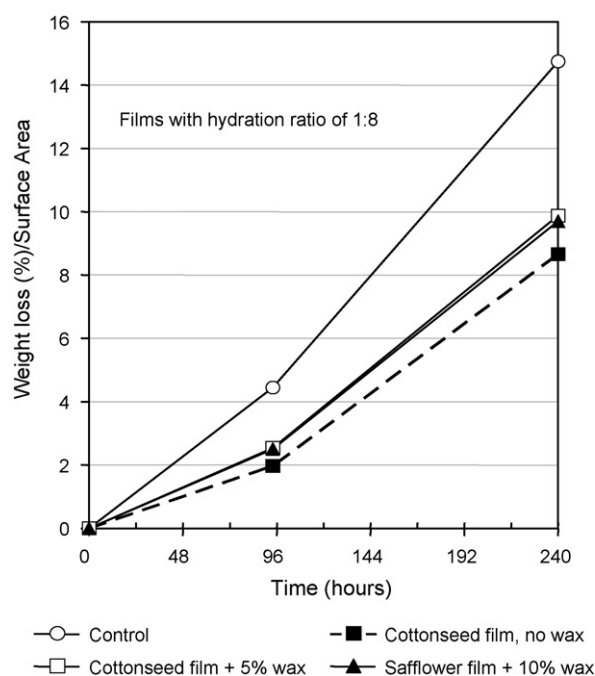
Fig. 3. Weight loss (%) per unit surface area (SA) in 'Camelot' bell peppers coated with oilseed-derived lipid films with paraffin wax.

Table 2
Summary of ANOVA for 'Camelot' bell peppers weight loss with oilseed-derived lipid films at 94 and 240 h.

Source	SS	df	MS	F	p-Value
94 h					
Treatment	17.65	3	5.89	6.84	<0.002
Error	21.50	25	0.44		
Total	39.15	28			
240 h					
Treatment	108.94	3	36.31	2.96	0.053
Error	294.7	24	12.28		
Total	403.67	32			

According to an ANOVA, the treatment mean and the standard deviation (SD) values of the weight loss per unit SA were only used at these benchmark points since the treatment mean values between and around the benchmark points were fairly representative (data not shown). Subsequently, the rest of the data were not presented. The results of an ANOVA (Table 2) indicated that there were statistically significant differences between the estimated values of the four groups at 94 h ($F_{3,24} = 6.844$; $p = 0.002$) and 240 h ($F_{3,24} = 2.957$; p 0.0053). The confidence level in the statistically estimated values of the data were shown by p and F values at 94 and 240 h. However, there were no significant differences between the films containing wax versus the cottonseed soapstock-derived film without wax.

The amount of wax added to the film was negatively correlated with weight loss, when the average weight loss values of the two groups, 0 and 5% wax, were compared with the control group. The same general trend of retarding weight loss was found with the lipid film from safflower containing 10% wax. Nevertheless, the rate of retardation in the weight loss with lipid film from safflower containing 10% wax was observed to be less compared with both the 100% lipid film derived from cottonseed containing no wax, and the cottonseed film containing 5% wax. The 100% lipid film derived from cottonseed containing no wax performed best in retarding the weight loss among the tested films. The film with 10% wax was prepared with the safflower soapstock whereas the films with 0 and 5% wax were from cottonseed-derived soapstock. It appears that adding 10% of paraffin wax to the gel of safflower oilseed lipids did not positively contribute to the formation of uniform lipid surfaces, possibly due to the difference in the physical properties of wax and oilseed lipids. For example, paraffin wax has a high melting point and a tendency to form a crystalline structure at room temperature (Small, 1986).

Theoretically, the safflower-derived film with twice the added parafilm should have performed better compared with the 5% wax addition in cottonseed-derived films. Even though the cottonseed lipid content is superior due to less unsaturated fatty acids, we speculate that the 5% cottonseed versus 10% safflower plus wax films achieved almost the same water retention results because the permeability of a paraffin wax increases 100 times when the paraffin oil content increases from 75 to 100% (Martin-Polo et al., 1992). Also, the complex mixture of alkanes in paraffin likely masked defects in the lipid crystallization by filling the cracks due to the retraction during the crystallization of the high solid content alkanes (Morillon et al., 2002).

The addition of wax to the cottonseed-derived films decreased the water vapor permeability slightly, as evidenced by less moisture loss, similar to previous reports where various waxes have been added to whey protein lipid-based films (up to a 35–40% concentration) (McHugh and Krochta, 1994; Shellhammer and Krochta, 1997). The permeability of a hydrogenated cottonseed oil based film increases 300 times as the liquid fraction of the lipid varies from 0 to 40% (Landmann et al., 1960). It appears that the solid or liquid state of lipid compounds, in conjunction with wax addition, strongly

loss were observed. Polynomial equations used to calculate weight loss per SA demonstrated a high degree of correlation regarding time-dependent effects. The 1:4 film polynomial equation was: $y = -0.0003x^2 + 0.0905x$ ($R^2 = 0.986$), whereas the equation for the 1:8 film was: $y = -0.0005x^2 + 0.2031x$ ($R^2 = 0.976$). The safflower-derived soapstock film resulted in the least effective water retention of the films and ratios tested, with roughly 21–25% reduction in weight loss per SA compared to the controls. Unsaturated fatty acids are less efficient to control moisture migration because they are more polar than saturated lipids. Films containing stearic or palmitic acids had better performances to reduce desiccation of oranges compared with similar coatings composed of oleic acid (Hagenmaier and Baker, 1997). Taking into consideration the relative percentages of saturated versus unsaturated fat in the two soapstocks (Table 1), our data are therefore in agreement with previous findings (Hagenmaier and Baker, 1997; Morillon et al., 2002). The safflower-derived films had much higher proportion (9% more) of unsaturated lipids than cottonseed-derived films, and were subsequently less effective in restricting water loss. The consistency of soapstock-derived lipid films was demonstrated by producing films from two different batches of cottonseed soapstock (CS-1 and CS-2) with a hydration ratio of 1:8. The resulting water loss profiles in bell peppers treated with these two films were almost identical (Fig. 2).

Edible films, especially including natural waxes, have been used for decades on fresh produce to create a semipermeable membrane on the surface to suppress respiration, control moisture loss, and more recently, to provide a delivery mechanism for the addition of functional components. We also subsequently tested water loss in films produced with a 1:8 hydration ratio plus and minus paraffin wax. In this analysis, peppers that remained at ambient temperature for 2 days prior to the application of film coating were used. A clear water loss trend and the pronounced effects between the control group and the group with the 100% oilseed-derived film (i.e. no wax) are presented in Fig. 3. Average weight loss in peppers coated with the 100% cottonseed soapstock-derived materials without wax was consistently about 1/3 less than the control group (Fig. 3). Weight loss data per unit SA versus time were presented at the two benchmark points, 4 days (94 h) and 10 days (240 h).

influences the barrier efficiency of the derived soapstock-derived film.

4. Conclusion

Soapstock is formed by treating vegetable seed oils with alkali, resulting in an oily matrix (roughly 10% or more crude oils mass) that has several non-oil components (Dowd, 1996). Oil-derived soapstocks are an underutilized agricultural byproduct. Although soapstocks are an underutilized agricultural byproduct, several cleanup steps including chemical modification would likely be required to attain food grade status. Nonetheless, it is clear from data presented herein that the soapstock-derived films significantly extended the shelf life of bell peppers. They effectively retarded the transfer of water vapor across the produce skin, which is the main cause for the postharvest losses.

The traditional shelf life extender using pure paraffin leaves a waxy residual feel and mouth-taste on cucumbers and bell peppers, even after water washing. Compared to the wax coat, the lipid films derived from oilseed soapstock can easily be removed from produce by simple water washing. Under alkali conditions and/or with water washing before consumption, potential residual gossypol in cottonseed, which is favored in an acidic environment, might not be of concern. Subsequently, other seed oils (e.g. safflower) should be explored further since cottonseed soapstock-derived films would likely not be advisable because some individuals have potential allergenicity issues relating to gossypol. Considering the advantage of the lipid film over the wax coat, use of the oilseed-derived biodegradable films appears to have a strong potential compared to presently used chemicals (Baldwin et al., 1997; Revathy and Narasimham, 1997), or wax (Petracek et al., 1998) as a shelf life extender. Further investigation, including more detailed appraisal of the postharvest physiological responses with the application appears to be worthwhile. Nonetheless, this preliminary data illustrating reduced water loss and shelf life preservation in bell peppers suggest that viable commercial films could be produced from underutilized agricultural soapstock byproducts.

Acknowledgments

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